

presumably normal Boxer sequence (Table 2). We hypothesized homozygosity for the NCL-affected PON but observed heterozygosity for all SNPs (Table 2). Our data indicate that these polymorphisms in *CTSD* can be excluded as causative mutations for the NCL phenotype in Tibetan Terrier and PON dogs.

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Assignment of 12 genes to porcine chromosome 1 by linkage and radiation hybrid mapping

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Source/description: In this study, 12 genes located in regions of human chromosomes 6, 9 and 15, which are homologous to quantitative trait loci (QTL) intervals on swine chromosome 1 (SSC1) (<http://www.animalgenome.org/QTLdb/>), were mapped on the Meat Animal Research Center (MARC) linkage map, the INRA-University of Minnesota porcine radiation hybrid (IMpRH)₇₀₀₀ panel,¹ or both.

Primer design and PCR conditions: Genomic DNA was amplified and sequenced with primers designed using Primer3 software (code available at: http://www-genome.wi.mit.edu/genome_software/other/primer3.html) to target introns and 3'-untranslated regions (UTRs) from porcine expressed sequence tags in the TIGR Pig Gene Index (Table 1). Reactions were performed in a PTC-225 DNA thermal cycler (MJ Research Inc, Watertown, MA, USA) using 0.3 U HotStarTaqTM polymerase (Qiagen, Valencia, CA, USA),

100 ng genomic DNA from eight parents of the MARC swine mapping family, 0.8 µM of each primer and 200 µM dNTPs in 25-µl reactions. Sequences and polymorphisms that were identified have been submitted to GenBank (Table 1).

In order to map genes with the 118-clone IMpRH panel,¹ we used primers designed as described above or from intron sequences generated in this study (Table 1). Amplifications were performed in 15-µl reactions in duplicate using 12.5 ng panel DNA, 1.5 mM MgCl₂, 200 µM dNTPs, 1 µM of each primer, 0.25 U HotStarTaqTM and 1X of supplied buffer. The PCR mixture was held at 94 °C for 15 min, cycled 45 times at 94 °C for 20 s, 57 °C for 30 s and 72 °C for 1–1.5 min, and then extended at 72 °C for 5 min. One-half of each reaction was analysed on a 2% agarose gel and manually genotyped.

Chromosomal location/linkage and radiation hybrid mapping: Amplicons that contained single nucleotide polymorphisms heterozygous in the common sire, or in at least two of the seven dams, were genotyped across the reference family using a primer extension assay on the Sequenom Mass-ARRAYTM system (Sequenom, San Diego, CA, USA). Linkage analyses were performed for the SNPs using seven families (86 progeny) of the MARC swine reference population.² Multipoint locations for mapped markers were based on the latest published swine genetic map (<http://www.marc.usda.gov/>). Seven genes with human homologues on chromosomes 6 and 9 (*SMPDL3A*, *RPL24*, *CREB3*, *AMBP*, *PAPPA*, *PTGS1*, and *SLC27A4*) were linkage mapped to SSC1 using these SNPs (Table 1).

In addition, radiation hybrid data for eight genes with human homologues on chromosomes 6, 9 and 15 (*RPL24*, *NTRK3*, *TMEM2*, *PTGS1*, *CIZ1*, *SLC27A4*, *GFI1B* and *AGPAT2*) were analysed for two-point and multipoint linkage with the IMpRH mapping tool and submitted to the IMpRH database (<http://imprh.toulouse.inra.fr/>). CarthaGene (<http://www.inra.fr/bia/T/CarthaGene/>) was used to estimate multipoint marker distance and order using all public markers on chromosome 1 in the IMpRH database (<http://imprh.toulouse.inra.fr/>) and those developed in this study. Five of the eight genes were assigned to SSC1 by radiation hybrid analysis, whereas three genes (*RPL24*, *PTGS1* and *SLC27A4*) were mapped to SSC1 by both linkage and radiation hybrid analyses (Table 1).

Comments: Several QTL for carcass and reproductive traits have been identified on the long arm of SSC1.^{3–6} Previous comparative mapping and chromosome painting⁷ analyses have shown that SSC1 is homologous to human chromosomes 6, 9, 14, 15 and 18. Mapping of these 12 genes to SSC1 is consistent with the homologous chromosomal regions previously reported,^{8,9} with rearrangements of human chromosome 9 (70 and 36 Mb) and chromosome 15 (83 Mb) corresponding to 56–96 cM on SSC1 (Table 1).

Table 1 Results for genes assigned to porcine chromosome 1 (SSC1).

Gene symbol	GenBank accession no. ¹	Forward primer (5' → 3')	Reverse primer (5' → 3')	Amplicon size (bp)	Map ²	Human position ³	Most significant marker	2-pt LOD	SSC1 position (cM) ⁴
<i>SMPDL3A</i>	DQ092608	tgtataaatgacaatttcaacaagag	ccagtgttaactgtgatgagaca	450	L	6:123	SW2130	11.35	48
<i>RPL24</i>	BV102946	cagtttcagcgggtacaaga	gagcagaaaccttcacaggc	400	R,L	9:70	SW781	32.21	56
<i>NTRK3</i>	BV103067	ctctattgcatcagtgccga	gtccagtgacgatccagtc	850	R	15:83	SW1431	8.90	82
<i>TMEM2</i>	DQ092607	gtgggggatacagtgctaagtc	ttctacagaaacggcaactt	500	R	9:72	S0155	7.67	94
<i>CREB3</i>	BV103209	cagaatctggagctacagaac	accaagatgcaggtgctgctg	1000	L	9:36	SW1092	27.29	96
<i>AMBP</i>	BV103453	aagaggaaggatcaggagctg	tccttctccgagcgaagttat	1200	L	9:112	S0354	31.71	107
<i>PAPPA</i>	DQ092606	cagtgccctgaatcacactac	cgttcatagttcagtcctatgt	600	L	9:116	SW1957	18.66	110
<i>PTGS1</i>	DQ092609	ctttctccacttctctgctgac	gccgcatctgcaacctacacca	360	R,L	9:121	S0112	21.97	121
<i>CIZ1</i>	BV103601	aaaggctcggagacctacagtc	tgatggcacaccggcggtctac	800	R	9:126	SW705	7.59	122
<i>SLC27A4</i>	BV103614	gctgtccgtgtcaacgaggac	gtcctctggacgatggtgccc	850	R,L	9:127	S0056	8.13	125
<i>GFI1B</i>	DQ092610	ggaccaggtcctgagcaacagc	gtgagtcggaagatggcgagtc	700	R	9:131	SSC10D08	11.64	136
<i>AGPAT2</i>	BV102613	acgtgggcgagcgcgatg	cgtgctgatgccagaaggagtg	150	R	9:135	SSC10D08	9.92	136

¹GenBank accession numbers refer to sequences obtained during the study and the single nucleotide polymorphisms are identified in those entries.

²Markers placed on the INRA-University of Minnesota porcine radiation hybrid₇₀₀₀ radiation hybrid map are designated with an R and those assigned on the Meat Animal Research Center (MARC) linkage map with an L.

³Chromosome: megabase position.

⁴Marker positions mapped on the radiation hybrid panel are estimated from microsatellite positions on the MARC linkage map.

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TLR4 variation in Yellowstone bison

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Source/description: Toll-like receptor 4 (TLR4) is a cell-surface receptor that activates innate and adaptive immune responses. Because it recognizes a broad class of pathogen-associated molecular patterns, including lipopolysaccharides, *TLR4* is a candidate gene for resistance to a large number of pathogens. Important examples are Gram-negative *Salmonella*,¹ *Pasteurella*,² and *Brucella*, as well as *Mycobacteria*,³ which include the causative agents of tuberculosis and Johne's disease.

Primer sequences: Primer sets originally designed for cattle *TLR4*⁴ were used to amplify and sequence bison *TLR4*, with the exception of one substitute forward primer (5'-CATTTTGGTTTCCTATTCAGCAG-3') that was used to sequence the 5'-end of exon 3.

Samples and sequencing: Genomic DNA samples were obtained from seven bison that wandered from Yellowstone National Park in different years and/or localities to minimize the possibility of sampling closely related individuals. Additionally, a portion of *TLR4* exon 3 was sequenced in 28 Yellowstone bison collected in 2002 to better estimate allele frequencies for two single nucleotide polymorphisms (SNPs).

Each exon was amplified twice for every individual. The separate replicates of each polymerase chain reaction (PCR) were used for sequencing in the forward and reverse directions, respectively, so as to reduce the risk of reporting